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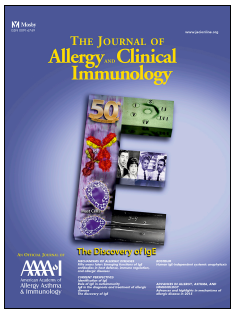

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PII: S0091-6749(16)30616-9

DOI: 10.1016/j.jaci.2016.04.062

Reference: YMAI 12225

To appear in: *Journal of Allergy and Clinical Immunology*

Received Date: 24 November 2015

Revised Date: 21 March 2016

Accepted Date: 30 April 2016

Please cite this article as: Jusot J-F, Neill DR, Waters EM, Bangert M, Collins M, Bricio Moreno L, Lawan Lawan K, Moussa Moussa M, Dearing E, Everett D, Collard J-M, Kadioglu A, Airborne dust and high temperatures are risk factors for invasive bacterial disease, *Journal of Allergy and Clinical Immunology* (2016), doi: 10.1016/j.jaci.2016.04.062.

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Airborne dust and high temperatures are risk factors for invasive bacterial disease

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Funding Sources: French Ministry of Foreign Affairs (FSP No. 2005–174) and Sanofi Pasteur (contract Men07) funded the climatic surveillance. Infection modeling was funded by the Institute of Infection and Global Health, University of Liverpool, UK.

Acknowledgements

Authors would like to address a special thank all the doctors and medical assistants who have sent CSF/TI specimens and epidemiological forms to the CERMES, and to the Direction de la Surveillance et de la Riposte aux Epidémies (DSRE) staff. We also specially thank CERMES' staff involved since 2002 in the meningitis surveillance and microbiology, especially Suzanne Chanteau and Pascal Boisier.

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Abstract

Background: The Sahel region of West Africa has the highest bacterial meningitis attack and case fatality rate in the world. The impact of climatic factors on patterns of invasive respiratory bacterial disease is not well documented.

Objective: We aimed to assess the link between climatic factors and occurrence of invasive respiratory bacterial disease in a Sahel region of Niger.

Methods: We conducted daily disease surveillance and climatic monitoring over an 8-year period between 1 January 2003 and 31 December 2010 in Niamey, Niger in order to determine risk factors for bacterial meningitis and invasive bacterial disease. We investigated the mechanistic effects of these factors on *Streptococcus pneumoniae* infection in mice.

Results: High temperatures and low visibility (resulting from high concentrations of airborne dust) were identified as significant risk factors for bacterial meningitis. Dust inhalation or exposure to high temperatures promoted progression of stable asymptomatic pneumococcal nasopharyngeal carriage to pneumonia and invasive disease. Dust exposure significantly reduced phagocyte-mediated bacterial killing and exposure to high temperatures increased release of the key pneumococcal toxin pneumolysin via elevated bacterial autolysis.

Conclusion: Our findings show that climatic factors can have a substantial influence on infectious disease patterns, altering density of pneumococcal nasopharyngeal carriage, reducing phagocytic killing, and resulting in increased inflammation and tissue damage and consequent invasiveness. Climatic surveillance should be used to forecast invasive bacterial disease epidemics and simple control measures to reduce particulate inhalation may reduce the incidence of invasive bacterial disease in regions of the world exposed to high temperatures and increased airborne dust.

Key messages

- Temperatures over 39.5C and increased airborne dust are significant risk factors for invasive pneumococcal diseases such as pneumonia and meningitis.
- Exposure to high temperatures and inhalation of airborne dust particulates drives progression from stable nasopharyngeal carriage to pneumonia and invasive disease.
- High temperatures and inhaled airborne dust particulates alter the functional activity of host immune cells and promote expression of bacterial virulence factors leading to increased pathogenicity.
- Limiting exposure to airborne dust in populations with high pneumococcal carriage rates will reduce the risk of invasive disease.

Key Words

Meningitis, Climate, *Neisseria meningitidis, Streptococcus pneumoniae*, Pollution, Dust

ABBREVIATIONS

A600: Absorbance, 600 nm

ANOVA: Analysis of variance

BHI: Brain heart infusion

CFU: Colony forming unit

CV: Coefficient of variation

ELISA: Enzyme linked immunosorbent assay

IQR: Interquartile range

IVIG: Intravenous immunoglobulin

MIP-2: Macrophage inflammatory protein 2

NaOH: Sodium hydroxide

Nm: *Neisseria meningitidis*

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OD: Optical density

OPKA: Opsonophagocytic killing assay

PBS: Phosphate buffered saline

PCR: Polymerase chain reaction

PLY: Pneumolysin

RH: Relative humidity

rpm: Revolutions per minute

RR: Relative risk

SEM: Standard error of mean

ST: Sequence type

T: Temperature

WHO: World Health Organisation

CAPSULE SUMMARY

Particulate pollution and extreme temperatures can influence nasopharyngeal bacterial carriage and predispose to invasive infection. Climatic factors likely influence bacterial disease incidence in areas of high temperature and dusty winds such as the African meningitis belt.

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Introduction

The 1,000 km wide semi-arid Sahel region, which lies between the Sahara desert to the north and the Sudanese Savanna to the south, has the highest attack rate (10 per 100,000) and case fatality rates (15%) in the world for bacterial meningitis[1,2]. This region, also known as the meningitis belt, comprises 350 million people at risk across 21 countries.

Niger, a Sahel country, has a long history of meningitis epidemics, with recent large-scale outbreaks occurring in 2000, 2003, and 2009. *Neisseria meningitidis* serogroup-A and X and *Streptococcus pneumoniae* are the main causative agents[3,4]. Meningitis outbreaks in Niger show strong seasonality, suggesting climatic factors could play a role in disease mechanisms[5-10], but these studies focus on all-cause meningitis and little is known about the specific impact of climate on bacterial meningitis.

The dry and dusty Harmattan winds that blow between November and May are a unique defining feature of the West African climate and have been associated with outbreaks of meningitis[11]. On its passage over the desert, the Harmattan wind picks up fine fractions of Saharan dust particles (mostly particulate matter <10 µm)[11]. The sheer amount of dust in the air can severely limit visibility and sometimes block the sun for several days, comparable to a heavy fog. Indeed, the inverse correlation between visibility and particulate matter concentration has been demonstrated in Niger and elsewhere[12, 13]. Dust is thought to have a negative impact on health, increasing morbidity due to diseases of the upper and lower respiratory tract[14]. A recent study, using a global atmospheric chemistry model, has suggested that outdoor air pollution leads to 3.3 million premature deaths per year worldwide with natural sources of particulate material (predominantly desert dust) responsible for 600,000 (18%) of those deaths[15]. In large parts of North and East Africa, the Middle East,

Central Australia and Central Asia, natural sources of small particulate material such as desert dust make a larger contribution to mortality than more recognized pollution sources such as industry, traffic, energy and agriculture. Thus, understanding the link between desert dust inhalation and mortality, and the climatic factors that influence levels of airborne dust, is key to disease control in affected areas.

Long-term forecasting and identification of climatic risk factors would help public health decision makers improve early warning systems and would help the scientific community to identify physiological factors implicated in the development of invasive diseases. Statistical forecasting models that integrate climatic factors, linking environmental and epidemiological surveillance, could act as early warning systems of infectious disease epidemics. Here we present findings from a study quantifying, on a daily-scale, this link between climate and meningitis in Niamey, Niger. Furthermore, we model these effects *in vivo* using experimental infection of mice.

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Methods

Ethics Statement

The biological surveillance was performed by the national reference centre of the Public Health Ministry of Niger, CERMES (Centre de Recherche Médicale et Sanitaire), which is part of the meningitis national control programme.

Study area and meteorology

The study area was defined as a radius of 50 km around the meteorological station of the international airport of Niamey, Niger and constituted a homogeneous geographical area for which climatic factors were measured daily. These measures comprise minimal and maximal temperatures, minimal and maximal relative humidity, mean wind speed, mean visibility (defined by the World Meteorology Organization as the maximal distance from which an observer can distinctly see an object on a horizontal plane), and rainfall. Seasons were defined by the National Forecasting Direction (Direction de la Météorologie Nationale) of Niger.

The population of the study area was 1,099,057 for the median year 2006. Cases of meningitis are registered daily and all cases within the study area confirmed by culture and/or PCR were enrolled between 1 January 2003 and 31 December 2010. Thirty-four health care facilities were involved. Full details can be found in the Online Supplement.

Mouse model of *Streptococcus pneumoniae* infection

All animal experiments were performed at the University of Liverpool, UK in accordance with the Animal Scientific Procedures Act 1986 and with the prior approval of the UK Home Office (PPL 40/3602) and the University of Liverpool ethics committee.

Sex- and age-matched MF1 mice (Charles River, UK) were used. Asymptomatic nasopharyngeal carriage was established in mice by intranasal infection, as described previously[16,17]. For particle inhalation experiments, two days post-infection, mice were given intranasal administration of 50 mg/ml silicon dioxide (dust, mean particle size 10 µm, Sigma, UK) or PBS as a control. This was repeated at four days post-infection and mice were culled at seven days post-infection or if invasive disease signs (as described by the scheme of Morton) progressed to visible lethargy[18]. For heat exposure experiments, mice were put in a heat box at 40°C for 10 minutes prior to, and for 20 minutes following, induction of nasopharyngeal carriage. Control mice were housed at 21°C throughout. Nasopharynx, lungs, brain and blood were removed and homogenised in PBS prior to plating on blood agar for assessment of tissue CFU. Full details can be found in the Online Supplement.

Pneumolysin detection ELISA

Sandwich ELISA was performed with mouse anti-pneumolysin (PLY) [PLY-4] (Abcam) and rabbit anti-PLY antibody (Abcam). Absorbance at 405nm was read using a Multiskan Spectrum microplate reader (Thermo Scientific). Full details can be found in the Online Supplement.

Opsonophagocytosis killing assay

Opsonophagocytic assay (OPKA) were performed as described[19], with minor modifications. Briefly, J774 mouse macrophages or HL-60 human neutrophils were incubated with 50µg/ml silicon dioxide for 1 hours shaking (175rpm) prior to addition of opsonized *S. pneumoniae* and complement. CFU were determined following a further 45 minutes (HL-60) or 60 minutes (J774) incubation. Full details can be found in the Online Supplement.

Measurement of autolytic activity

Triton X-100 induced autolysis assays were performed as described by Houston et al.[20] Full details can be found in the Online Supplement.

Haemolytic assay

Overnight cultures of *S. pneumoniae* serotype-2 (strain D39) and its isogenic autolysin (LytA)-deficient mutant were subcultured in BHI media and incubated at 37°C or 40°C to A600=1.0. Cells were then pelleted and the supernatant removed and filter sterilised. Hemolytic activity against sheep red blood cells was measured as described previously[21].

Statistical analysis

A descriptive analysis was performed for the median and the interquartile range of the climatic factors, with the range and coefficient of variation according to season. A Mantel-Haenszel chi-squared test was used to adjust the relative risk for a maximal temperature threshold over 39.5 °C on seasons with the statcalc program of Epiinfo 6.04 software.

To regress a time-series of daily counts of confirmed cases of meningitis with daily changes in climatic factors, a generalized additive model with a negative binomial family was used. Full details can be found in the Online Supplement. All analyses were performed with R software (R Development Core Team, 2010), version 2.12.0.

Mouse model data were analysed in Graph Pad Prism using ANOVA or logrank test with appropriate post testing. Results with p-values less than 0.05 were considered significant. Data represent mean +/- SEM unless otherwise indicated. Data were assessed for normality using the D'Agostino-Pearson (omnibus K2) test.

Author contributions

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JFJ and DRN contributed equally to the study. JFJ built the methodology of the epidemiological study and analysed the data. KLL and MMM defined the study area and collected environmental data. DRN, MB, LBM, and MC performed the *in vivo* experiments. EMW and ED performed the *in vitro* experiments. DRN, JFJ, EMW, DE, JMC and AK analysed the data. DRN, JFJ, JMC and AK wrote the manuscript. JFJ designed the epidemiological study and JMC coordinated the microbiological analyses in Niger. DRN and AK designed *in vitro* and *in vivo* experiments. JMC and AK coordinated and led the study equally throughout.

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Results

We conducted daily disease surveillance and climatic monitoring over an 8-year period between 1 January 2003 and 31 December 2010 in Niamey, Niger. Over the 8 years, 893 confirmed cases of bacterial meningitis were recorded within the study site. Epidemics ranged in size from 36 in 2008 to 305 in 2006, with corresponding attack rates of 3.3–27.8 10^{-5} (Table 1).

Children under 15 years of age were the most severely affected age group, accounting for 81.7% of cases. *S. pneumoniae* was the major cause of meningitis epidemics in five of the eight years and was responsible overall for 25.9% of total cases (Figure 1). *N. meningitidis* was the other predominant causative agent, particularly during epidemics, and serogroups-X (50.1% of total *N. meningitidis* cases) and A (33.0%) were common (Figure 1).

Climate monitoring demonstrated that all factors other than wind speed displayed strong seasonality (Figure 2 and Table 2). High maximal temperatures over 40°C were observed in all seasons and minimal temperatures over 30°C were recorded during the very hot and rainy seasons. The most striking associations between climatic factors and meningitis cases were increased meningitis cases with increasing maximal temperature, low visibility, and low maximal relative humidity (Figure 2).

Highest numbers of meningitis cases were recorded from a threshold maximum temperature of 39.5°C ($\beta = 0.087$, SE = 0.042, $p = 0.04$), with an excess risk of 9.1% for an increase of 1°C (Figure 3) and this risk could not be explained by seasonal variation in incidence alone (Table 3).

An increase in visibility from 0.3 to 5.3 km led to a decrease in the number of meningitis cases (β = -0.49, SE = 0.15, p = 0.001) 34–44 days later. Five days after an increase in maximal relative humidity from 38% to 72%, the number of meningitis cases decreased (β = -1.86, SE = 0.69, p = 0.007).

Decreased visibility is predominantly the result of increased airborne dust and so a potential explanation for the association with increased incidence of meningitis is that inhalation of particulate matter during periods of low visibility increases the susceptibility of individuals to developing invasive bacterial disease. We tested both this hypothesis, and the association of temperatures above 39.5°C with invasive bacterial disease, in a model of pneumococcal nasopharyngeal carriage. In this model, pneumococci stably colonise the naso-oropharynx and carry for long periods with no invasion into the lower respiratory tract and no transmission into blood[16, 17]. Thus, this system models the situation in Niger, where a high proportion of children have asymptomatic nasopharyngeal colonisation with potentially pathogenic bacteria including *S. pneumoniae*, *H. influenzae* and *N. meningitidis*.

S. pneumoniae colonised mice displayed significantly increased densities of pneumococcal carriage in the naso-oropharynx following dust exposure as compared to normal bacterial colonization controls (Figure 4A). Importantly, this was accompanied by significant invasion of bacteria into lung and brain following dust exposure (Figure 4B and 4C). This was the case both for mice colonised with the laboratory serotype 2 strain of *S. pneumoniae* (D39) and those colonised with a clinical serotype 1 isolate (Figure 4). Serotype 1 *S. pneumoniae* isolates were frequently recovered from meningitis patients in our disease surveillance study (44.8% of *S. pneumoniae* cases).

High temperatures also emerged as a significant risk factor for bacterial meningitis, and we sought a direct demonstration of the effect of temperature on invasive bacterial infection. Mice were exposed to temperatures of 40°C (above the 39.5°C threshold) for 10 minutes before and 20 minutes after pneumococcal colonisation. Following heat exposure, pneumococcal numbers in the nasopharynx and brain remained comparable to controls (Figure 4A and C), but significantly increased numbers were recovered from the lungs (Figure 4B). This demonstrates that invasive dissemination from the nasopharynx to the lungs occurs following exposure to extreme temperatures. Mice exposed to both dust inhalation and high temperatures had significantly increased bacterial numbers in lung tissue compared to mice exposed to either dust or high temperature alone (Figure 4B).

The combinatorial effect of dust and high temperature was also evident in survival analysis of infected mice (Figure 4D). Visible disease signs and progression to death do not ordinarily occur in the pneumococcal nasopharyngeal carriage model and this was the case for the serotype 1 colonised mice and for the serotype 2 colonised mice that were not exposed to dust (Figure 4D)[16]. However, following dust exposure, 23% of serotype-2 colonised mice developed severe invasive disease and had to be culled (Figure 4D). Mortality increased to 54% when dust inhalation was combined with exposure to high temperatures (Figure 4D). All mice that died had significantly increased bacterial loads in their nasopharynx, lungs, brain, and blood compared to survivors (data not shown).

To explore potential mechanisms of dust- or temperature-induced susceptibility to pneumococcal disease, we examined a key component of innate antibacterial defence; phagocytic responses. We observed significantly increased levels of the neutrophil chemoattractant MIP-2 (Figure 5A) and elevated neutrophil numbers (Figure 5B) in lungs of

dust-exposed mice, as compared to PBS-exposed and naïve mice. However, surprisingly, increased infiltration of phagocytic cells did not lead to enhanced bacterial clearance (Figure 4A-C). To determine whether dust-exposed phagocytes were impaired in their ability to kill bacteria, opsonophagocytic killing assays were performed with untreated or dust-exposed neutrophils and macrophages (Figure 5C). Both macrophage and neutrophil cell lines showed a significantly decreased ability to kill pneumococci following dust-exposure (Figure 5C), suggesting that the increased recruitment of phagocytic cells into lungs in dust-exposed mice is ineffective in containment and clearance of pneumococcal infection.

Previous studies in *Staphylococcus aureus* have described temperature-dependent changes in the rate of bacterial autolysis[22]. We sought to determine whether the enhanced virulence of pneumococci at high temperatures might be due to increased autolysis, and thus increased release of the cytosolic toxic pneumolysin. Serotype 2 *S. pneumoniae* cultures were grown to OD_{600} 1.0 at 37°C or 40°C before addition of Triton-X to the cultures. Cultures that had been grown at 40°C displayed a markedly increased rate of autolysis as compared to those grown at 37°C (Figure 5D). Cell death was significantly reduced in cultures of autolysin-deficient serotype 2 pneumococci grown at either 37°C or 40°C (Figure 5D).

Importantly, increased autolysis was associated with increased release of pneumolysin into the culture medium (Figure 5E and F). Supernatant from 40°C cultures induced significantly greater lysis of erythrocytes than supernatant from 37°C cultures (Figure 5E) and 40°C supernatants contained on average more than 2-fold higher levels of pneumolysin than 37°C cultures of comparable optical density (Figure 5F). Thus, increased bacterial lysis and toxin release in the nasopharynx during periods of high temperature may damage the respiratory epithelium, allowing surviving bacteria a route by which to disseminate within the host and

may also lead to lysis of recruited host leukocytes, further impairing anti-pneumococcal immunity and hampering containment and removal of infection.

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Discussion

We have provided the first quantified risk of the occurrence of meningitis linked to climatic factors including high temperature, low visibility and dust. These data demonstrate that environmental exposure to inhaled particulates or extremes of temperature can significantly increase bacterial numbers in the respiratory tract and lead to invasive disease with increased risk of mortality via mechanisms including impaired phagocytic function and increased release of toxins.

The huge epidemic of *Neisseria meningitidis* serogroup X meningitis in and around Niamey in 2006 has been reported elsewhere[23]. Although rare, sporadic epidemics of serogroup X meningitis have occurred previously in Niger[24]. In all years other than 2006, numbers of meningitis cases caused by *N. meningitidis* and *S. pneumoniae* were comparable, together accounting for 79-96% of cases, with a small but consistent year-on-year contribution from *H. influenzae* (1-16%).

It is difficult to extrapolate data from a meteorological station to an entire district and therefore impossible to study the link between meningitis and climate without incurring ecological bias. To minimize this bias, daily changes in the count of clinical meningitis cases and climatic factors were obtained throughout the study period (8 years). Furthermore, reinforced microbiological surveillance since 2002 in Niger provides reliable daily counts of biologically confirmed cases of acute bacterial meningitis. Other studies have used data from epidemiological surveillance based on weekly collection of notifications of suspected meningitis cases at district level within a meningitis belt country. Consideration should be given to implementation of new models integrating climatic data with high-quality, case-based meningitis surveillance data (based on new WHO guidelines on meningitis outbreak

responses) across the African meningitis belt. This could expedite design of effective epidemic control strategies and aid management of risk. Dust exposure, for example, could be minimized with simple interventions such as the use of scarves around the nose and mouth during periods of low visibility.

Saharan dust, carried by the Harmattan, has been shown previously to affect health, particularly by exacerbating asthma and favouring the establishment of respiratory infections[25-27], and is thought to have contributed to meningitis outbreaks in Burkina Faso and Niger[3]. Previous studies have demonstrated that uptake of particulates by macrophages can disrupt phagocytic bacterial killing[28] and we demonstrate here that dust-exposed phagocytes (both macrophages and neutrophils) are functionally impaired. Thus, we propose that one mechanism underlying dust-induced disease susceptibility may be that inhalation of dust generates an inflammatory lung condition coupled with impaired phagocytic bacterial clearance, creating an environment conducive to bacterial survival and dissemination to sites such as the brain.

The ability of inhaled dust to drive up bacterial loads in the nasopharynx is significant, as we have recently described how changes in carriage density substantially affect the delicate balance of host immune control in the nasopharynx, driving immune-tolerogenic responses towards damaging pro-inflammatory ones as bacterial burden increases[17]. Inhaled dust is likely to trigger inflammatory reactions at the surface of the upper airway mucosal epithelium both by direct abrasion of the respiratory surface and due to its affect on bacterial carriage density. This increased inflammation could induce increased expression of host receptors that act as binding sites for bacteria[29]. Thus, by triggering local inflammation, inhaled dust may drive colonised bacteria towards a more invasive phenotype.

Set against a backdrop of accelerated climate change, high temperatures could have a strong future impact on the occurrence of bacterial meningitis. Extremes of temperature may cause heat stress in both pathogen and host and thereby favour transition from carrier state in the naso-oropharynx to invasiveness in part through the induction of synthesis of stressor-induced proteins that play a complex role in the phenotypic manifestation of virulence[30]. Furthermore, at high temperatures oxidative stress increases and antioxidants become scarce. Cellular oxidative stress is associated with impairment of host immunity and immune responsiveness has been found to correlate with levels of antioxidants in plasma in carriers of *N. meningitidis*, particularly in children under 3 years of age[31, 32].

Pneumolysin may also be key to the link between high temperatures and invasive bacterial disease. Pneumolysin is a key pneumococcal virulence factor and can both promote and dampen inflammation through its ability to induce host cell lysis at high concentrations as well inducing a wide range of effects at sub-lytic concentrations[29]. Pneumolysin-deficient pneumococcal strains are attenuated in virulence in animal disease models, including those of meningitis[33] and levels of pneumolysin in the CSF in meningitis correlate negatively with patient outcomes[34].

Our data demonstrated an interaction of heat and dust-inhalation, whereby mice exposed to both were at significantly increased risk of developing invasive pneumococcal disease. It may be that the combination of abrasion of the respiratory tract, impaired phagocytosis and increased release of damaging pathogen toxins creates a ‘perfect storm’ for dissemination of colonised bacteria from the nasopharynx. Alternatively, the effects of particulate inhalation[35] and high temperatures[36] on respiration may lead to direct aspiration of pre-colonized or

aerosolized bacteria into the lung. Further mechanistic studies in this area are urgently required to determine how climatic factors contribute to bacterial disease incidence during epidemics.

Collectively, these findings have significant implications for those areas of the world with high bacterial carriage rates coupled with hot climates and high levels of natural pollution. In such settings, high levels of atmospheric dust and increased temperatures combine to create a significant risk factor for the development of invasive disease.

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Table 1. Distribution of meningitis cases per year and according to season and threshold of maximal temperature

	2003	2004	2005	2006	2007	2008	2009	2010	2003–2010*
Annual cumulative incidence (cases)	148	93	84	305	44	36	123	60	112
Attack rate (cases per 100 000)	13.5	8.5	7.6	27.8	4.0	3.3	11.2	5.5	10.2
Cases during very hot season (%)	77.1	36.3	47.7	93.7	38.6	47.3	87.9	76.7	74.0
Cases when T_{max} ≥39.5°C (%)	35.8	33.3	47.6	89.5	29.5	36.1	82.9	45.0	62.5
Number of days when T_{max} ≥39.5°C	90	89	95	105	97	85	100	113	97

*: average of the parameters on the overall period

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Figure 1. Temporal changes in the causative agent and number of cases of bacterial meningitis in Niamey, Niger. Nm, *Neisseria meningitidis*

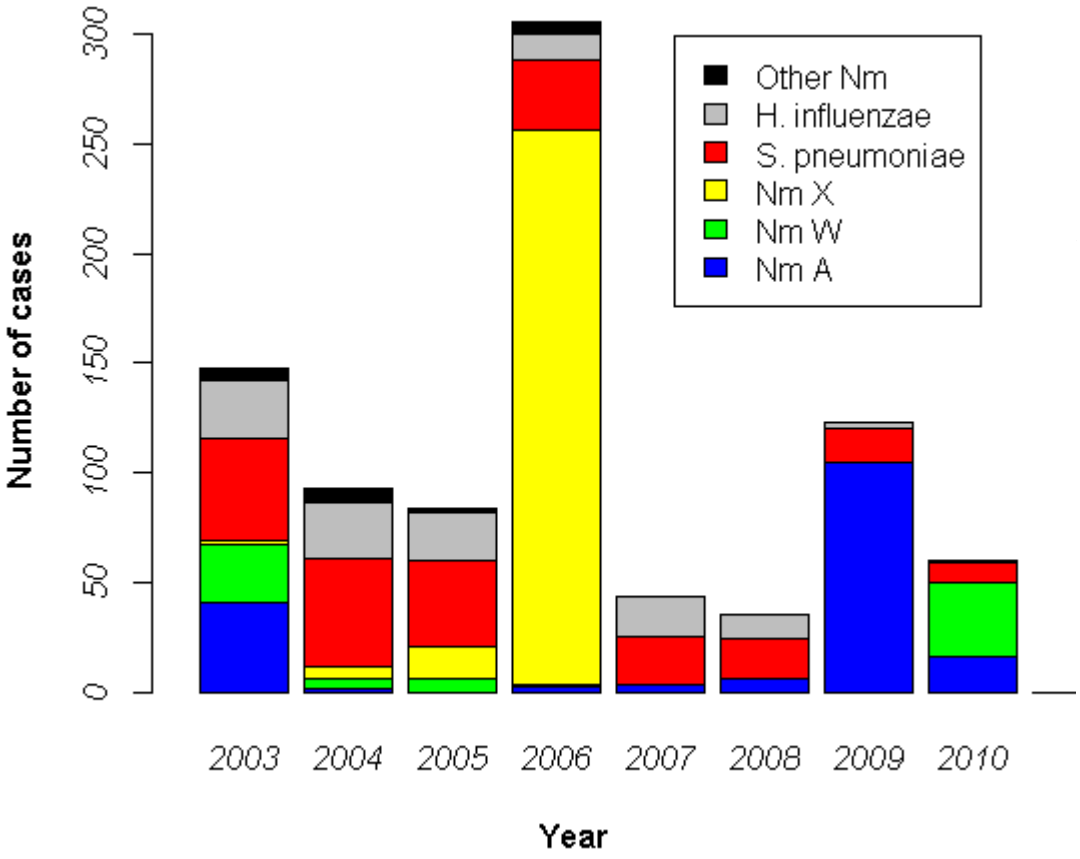

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Table 2. Climatic factors suspected to be linked with meningitis, by season.

T, temperature; RH, relative humidity; IQR, interquartile range; CV, coefficient of variation

Season	T max (°C)	T min (°C)	RH max (%)	RH min (%)	Rainfall (mm)	Wind speed ($m.s^{-1}$)	Visibility (km)
Cold							
Median (IQR)	34.1 (4.8)	17.4 (3.4)	32 (11)	10 (4.3)	0 (0)	6.4 (2.7)	5.4 (2.4)
Range	24.6–41.0	11.2–29.2	13.1–54.0	2–22	0–0	1.4–13.6	0.3–7.1
CV (%)	9.6	14.4	23.1	32.7	NC	31.0	35.6
Very hot							
Median (IQR)	37.5 (4.4)	26.5 (5.6)	33 (29)	12 (14)	0 (0)	6.4 (2.8)	5.4 (0.9)
Range	25.4–46.2	15.5–33.0	7.34–97.0	2–88	0–19	2.2–12.9	0.5–7.1
CV (%)	7.0	14.3	46.8	66.3	13.6	30.6	35.4
Rainy							
Median (IQR)	35 (3.5)	25 (3.5)	86 (16)	46 (17)	0 (0.2)	6.1 (2.8)	6.5 (0.6)
Range	23.7–43.5	18.0–33.5	32–100	10–80	0–7.3	1.8–13.6	3.6–8.5
CV (%)	9.3	9.8	13.8	25.8	2.5	31.2	8.0
Hot							
Median (IQR)	37.5 (3.5)	22 (5.6)	47 (27.2)	15 (11)	0 (0)	4.5 (2.0)	6.0 (2.6)
Range	27.5–41.2	11.6–29.0	20.6–100	5–59.2	0–6.2	1.6–9.8	0.9–8.0
CV (%)	6.8	15.4	32.1	54.6	10.0	30.5	20.6

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Figure 2. Temporal changes in number of meningitis cases with climatic factors in Niamey. T, temperature; RH, relative humidity. Visibility is defined as the maximal distance from which an observer can distinctly see an object on a horizontal plane.

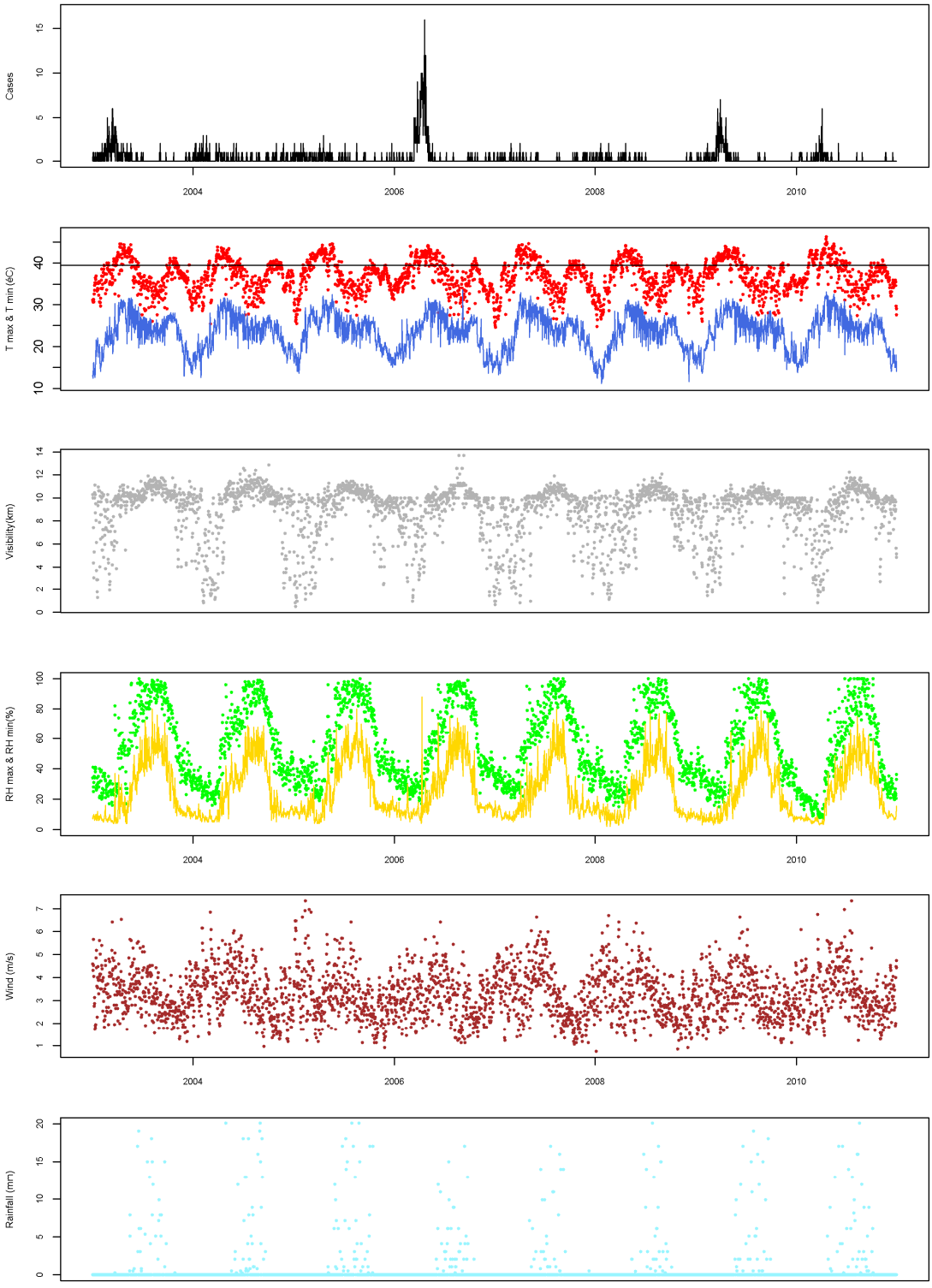

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Figure 3. Relative risks for meningitis by maximal temperature from a threshold of 39.5 °C. Gray zone corresponds to the 95% confidence interval of the risks above a maximal ambient temperature of 39.5°C. A significant impact was observed from a maximal ambient temperature of 39.5°C and no significant impact was found under this value.

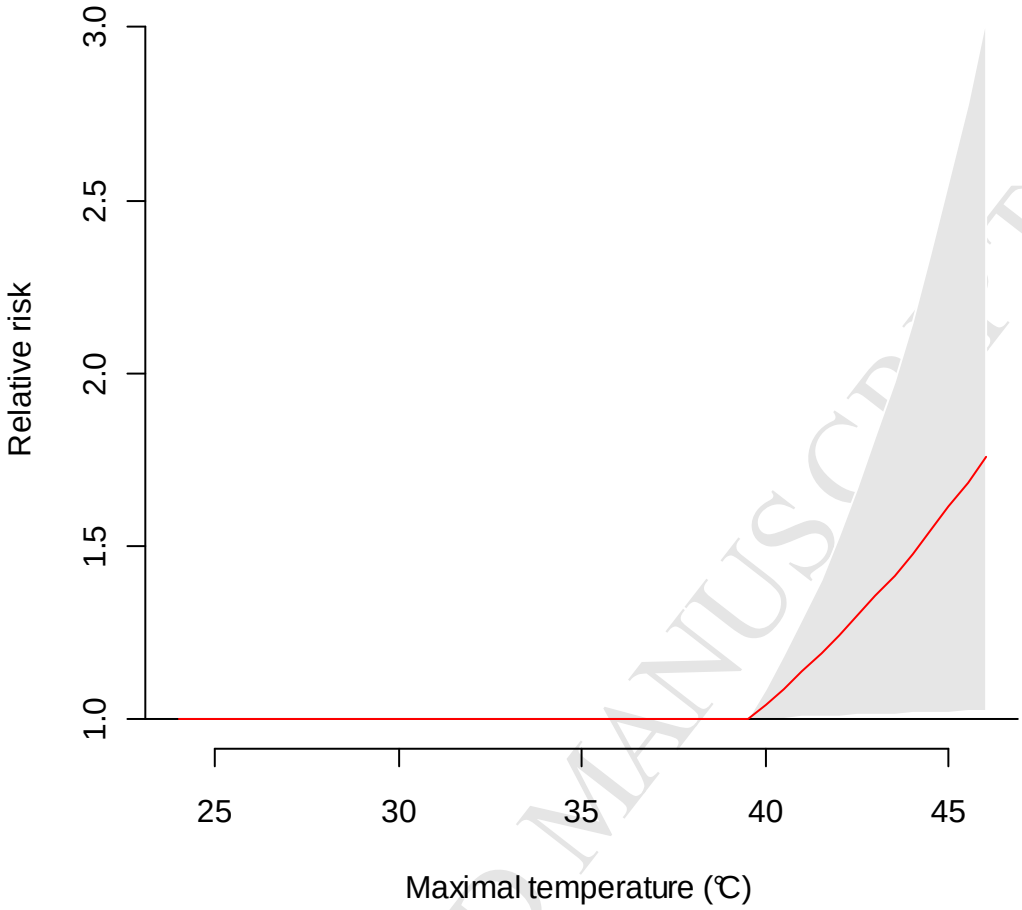

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Table 3. Risk for meningitis according to a threshold of maximal temperature ≥ 39.5°C, adjusted for season

	No. of days with at least one meningitis case when maximal temperature		No. of days with no meningitis case when maximal temperature		Relative risk	95% confidence interval
	≥ 39.5°C	< 39.5°C	≥ 39.5°C	< 39.5°C		
Season						
Rainy	8	52	78	837	1.59	0.78 – 3.24
Hot	8	36	91	465	1.12	0.54 – 2.35
Cold	5	101	4	378	2.63	1.43 – 4.85
Very hot	232	72	348	206	1.54	1.24 – 1.93
Temperature (crude RR)	253	261	521	1886	2.69	2.31 – 3.13
Temperature (RR adjusted for season)					1.54	1.26 – 1.88

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Figure 4. Inhaled dust and exposure to high temperatures increase invasiveness of *S. pneumoniae* in mice. Mice were colonised with *S. pneumoniae* serotype 2 strain D39 or a Niger serotype 1 meningitis isolate (ST303) and challenged intranasally with dust at 2 and 4 days post-colonisation. Mice were kept at room temperature (21°C) or 40°C as indicated for 10 minutes prior to and 20 minutes following infection and dust/PBS exposure. **(A-C)** Colony forming units (CFU) per mg tissue in **(A)** nasopharynx, **(B)** lungs and **(C)** brain at 7 days post-infection. **(D)** Kaplan-Meier survival curve. Asterisks represent significance in one-way ANOVA with Dunn's post-test **(A-C)** or logrank analysis **(D)**. * = p<0.0.5, ** = p<0.01, *** = p<0.001.

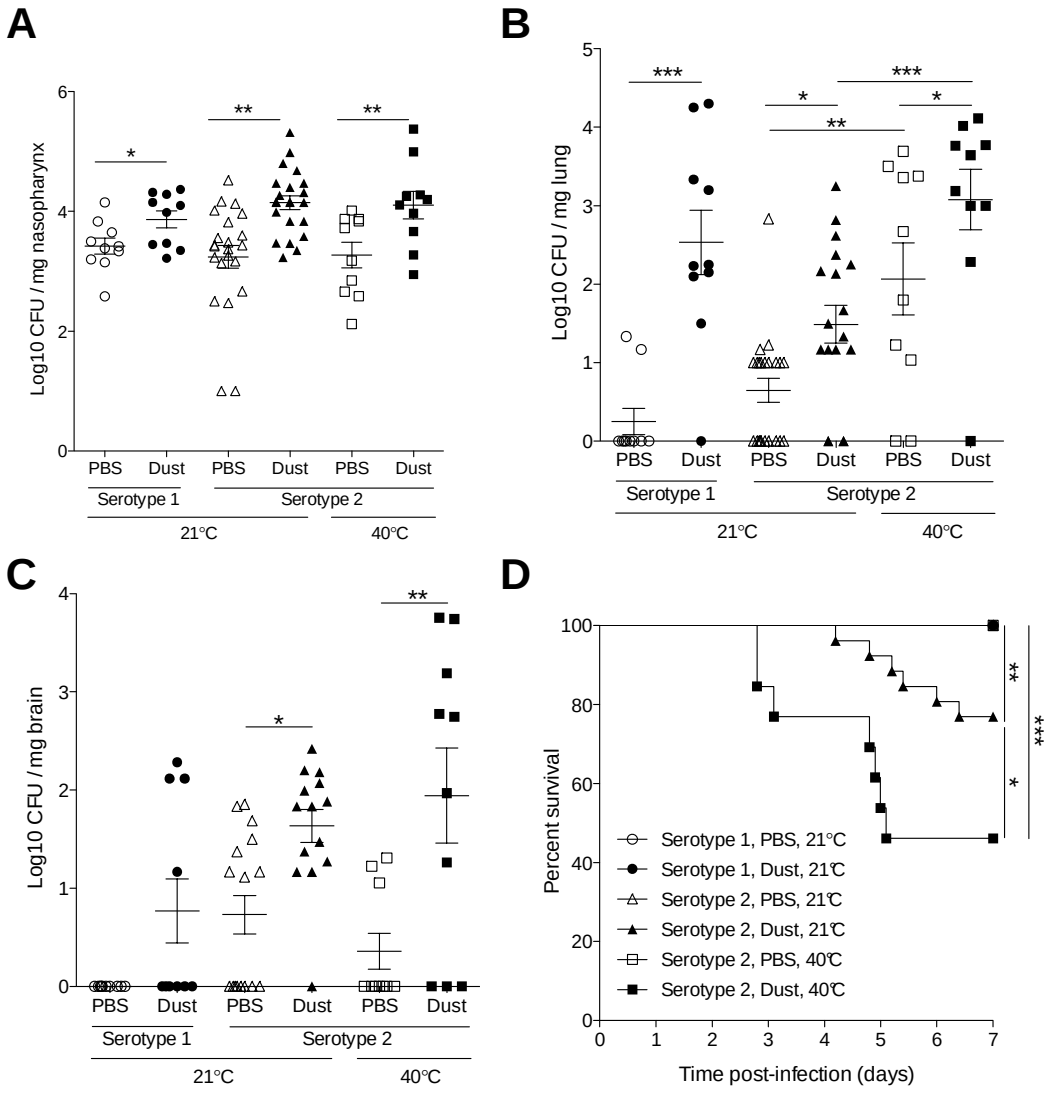

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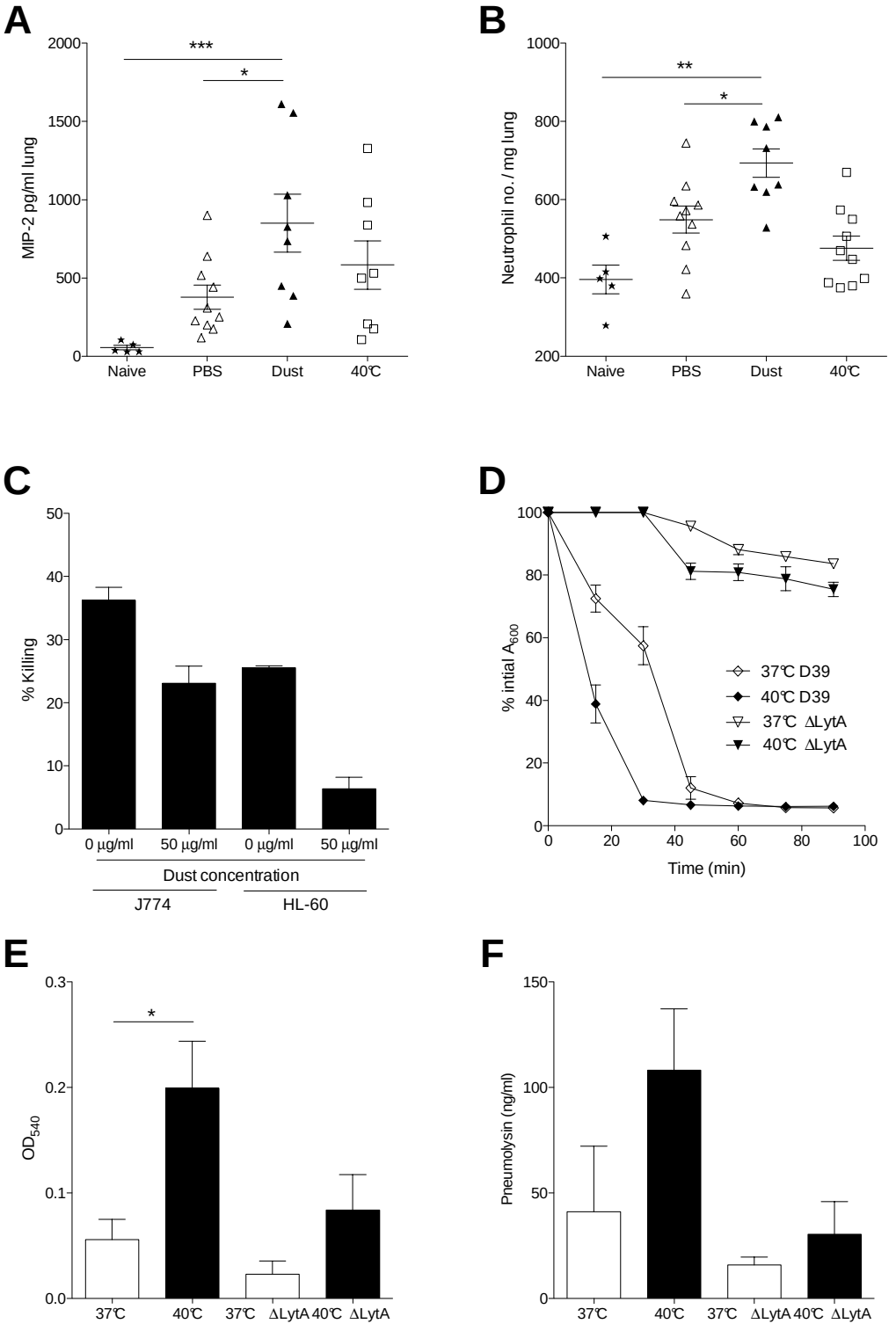
Figure 5. Dust exposure inhibits phagocytosis and high temperatures induce pneumococcal autolysis and pneumolysin release. (A) ELISA quantification of MIP-2 and **(B)** flow cytometric determination of neutrophil (Gr-1high) numbers in lungs at day 5 post-infection. **(C)** Killing (as percentage of total bacteria added) of D39 by J774 macrophages and HL-60 neutrophils with or without pre-incubation of phagocytes with dust. **(D)** Triton X-100-induced autolysis of serotype 2 (D39) and its autolysin-deficient Δ*lytA* strain grown at either 37°C or 40°C. **(E)** Hemolytic activity measured as increased optical density following lysis of sheep erythrocytes. **(F)** ELISA calculated pneumolysin concentration in filtered supernatant of D39 and Δ*lytA* grown at either 37°C or 40°C. until A600 = 1.0. Results are representative of three independent experiments and show mean +/- SEM. Asterisks represent significance in one-way ANOVA with Dunn's post-test. * = p<0.0.5, ** = p<0.01.



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Online Supplement: Methods

Study area and meteorology

The study area was defined as a radius of 50 km around the meteorological station of the international airport of Niamey, Niger. This choice was made in accordance with the National Forecasting Direction (Direction de la Météorologie Nationale) of Niger to obtain a homogeneous geographical area for which climatic factors are measured daily. These measures comprise minimal and maximal temperatures, minimal and maximal relative humidity, mean wind speed, mean visibility (defined by the World Meteorology Organization as the maximal distance from which an observer can distinctly see an object on a horizontal plane), and rainfall. Seasons were defined by the National Forecasting Direction (Direction de la Météorologie Nationale) of Niger.

The population of the study area was 1,099,057 for the median year 2006. Children aged between 0–5 years represented 21.9% of the population. As all cases of meningitis are registered daily, all cases of acute bacterial meningitis confirmed by culture or PCR occurring within the study area were enrolled between 1 January 2003 and 31 December 2010. Thirty-four health care facilities were involved in the survey. Almost all the suspected meningitis cases were referred to the national hospital of Niamey (72.3%). The other health care structures in Niamey that participated in the survey were the National Hospital of Lamordé, the La Poudrière Regional Hospital, and two health care centres located at the periphery (altogether 13.6% of the meningitis cases). The remaining cases were detected in one district hospital, six private clinics, and 22 health care centres. 68.2% of the total population was vaccinated on 20 April 2009 with a bivalent polysaccharide A/C vaccine following an outbreak involving *N. meningitidis* serogroup-A (vaccination coverage from 92 to

100% according to the district of Niamey in people aged 2–30 years). The vaccination coverage was assumed to decline linearly according to population growth at a daily rate of 0.02%.

Laboratory testing

Cerebrospinal fluid samples were analysed by PCR at the Niger National Reference Laboratory for bacterial meningitis, where all samples collected in the country are analysed[2]. A confirmed case of acute bacterial meningitis was defined as a first positive culture or PCR for *N. meningitidis*, *S. pneumoniae* or *Haemophilus influenzae*. For *N. meningitidis*-positive samples, a slide-agglutination with anti-sera or a second PCR was performed to identify serogroups A, B, C, X, Y, and W. The characteristics of the patient on the epidemiological form and the PCR results were recorded into a database.

Mouse model of S. pneumoniae infection

All animal experiments were performed at the University of Liverpool, UK in accordance with the Animal Scientific Procedures Act 1986 and with the prior approval of the UK Home Office (PPL 40/3602) and the University of Liverpool ethics committee.

Sex- and age-matched (8-10 weeks) MF1 mice (Charles River, UK) were divided into cages of equal size (usually three to five mice) on arrival by animal unit technical staff with no involvement in study design. For a single experiment, all mice were within 2 g (total range for study of 19-24 g) weight of each other. Investigators were

blinded to group allocation and unblinding was performed post-experiment, when bacterial numbers had been enumerated.

As described previously, asymptomatic nasopharyngeal carriage was established in mice intranasally infected with 1 x 10^5 colony forming units (CFU) of *S. pneumoniae* serotype-2 (strain D39) or a Niger serotype-1 strain (ST303) isolated from a child meningitis case[14,15] in 10 µl PBS. Infection was performed under light anaesthesia with O_2/isofluorane. For particle inhalation experiments, two days post-infection, mice were given intranasal administration of 50 mg/ml silicon dioxide (dust, mean particle size 10 µm, Sigma, UK) in 10 µl PBS (10 mice for serotype 1, 22 mice for D39) or PBS only as a control (10 mice for serotype 1, 22 mice for D39). This was repeated at four days post-infection and mice were culled at seven days post-infection or if invasive disease signs (as described by the scheme of Morton) progressed to visible lethargy[29]. For heat exposure experiments, mice were put in a heat box at 40°C for 10 minutes prior to, and for 20 minutes following, induction of nasopharyngeal carriage (10 mice). Control mice were housed at 21°C throughout (10 mice). Nasopharynx, lungs, brain and blood were removed and homogenised in PBS prior to plating on blood agar for assessment of tissue CFU (secondary outcome).

Pneumolysin detection ELISA

96 well ELISA microplates (Corning) were coated overnight at 4°C with 1µg/well mouse anti-PLY [PLY-4] antibody (Abcam). Following washing, plates were blocked for 2 hours, washed again and then 100µl bacterial culture supernatant was added for 2 hours. Following washing, 1µg/well rabbit anti-PLY antibody (Abcam) in 100µl

diluent was added for 2 hours. Plates were washed and goat anti-rabbit: alkaline phosphatase antibody (Abcam) was added for 30 minutes. After washing, 250µl/well pNPP colour reagent (Sigma Aldrich) was added for 15 minutes before the reaction was stopped with 50µl 3N NaOH. Absorbance at 405nm was read using a Multiskan Spectrum microplate reader (Thermo Scientific).

Opsonophagocytosis killing assay

Opsonophagocytic assay (OPKA) were performed as described[30], with minor modifications. Briefly, 1×10^5 J774 mouse macrophages or 4×10^5 HL-60 human neutrophils were incubated with 50µg/ml silicon dioxide (sand, mean particle size 10 µm, Sigma, UK) for 1 hours shaking (175rpm) prior to addition of 1×10^3 opsonized *S. pneumoniae* and complement. CFU were determined following a further 45 minutes (HL-60) or 60 minutes (J774) incubation. IVIG at a final dilution of 1:20 was used as the source of pathogen-specific antibody for opsonisation. Wells containing non-opsonised pneumococci were used as controls.

Measurement of autolytic activity

Triton X-100 induced autolysis assays were performed as described by Houston et al.[31] Overnight cultures of *S. pneumoniae* serotype-2 (strain D39) and it's isogenic LytA-deficient mutant were subcultured in BHI media and incubated at 37°C or 40°C to A600 = 1.0. Cells were then pelleted and washed twice with PBS and subsequently re-suspended in PBS containing 0.02% Triton X-100. The suspensions were then incubated at 37°C or 40°C. A600 readings were taken at 0 min then at 15 min intervals. Triton X-100 induced autolysis was measured as a percentage of the initial A600. Each experiment was repeated three times.

Statistical analysis: general additive model

The general model was written as follows:

$$Cases = \alpha + s(time) + \beta.s'(climatic\ factors) + \beta_t.t(climatic\ factors) + \gamma.factors,$$

The models were fitted by controlling for long-term trend and seasonality by a penalized thin-plate regression spline s with the mgcv package, which allowed optimization of the numerical method for smoothing and minimised autocorrelation in the residuals. *s'* was spline function, β the coefficient of the variable with non-linear effects and γ the coefficient of the linear predictor, such as season, weekdays, holiday, celebration or vaccination rate. The climatic factors minimal and maximal temperatures, minimal and maximal relative humidity, rainfall, wind speed, and horizontal visibility, were tested as independent covariates in the models. The natural history of meningitis often involves a period of nasopharyngeal carriage prior to invasion of meninges. The model aimed to explore two steps: immediate invasive meningitis following 0 to 5 days the exposure (very short term effect of environmental exposure) or acute meningitis after a period of carriage (up to 40 days) (short term effect of environmental exposure). A distributed lag model performed with the dlnm package was used, with a natural spline (s') to estimate the health impact of the climatic factors on the current day and several previous days. The distributed lag model allows the effect of a given day's increase in a given climatic factor to be distributed over several days after its values increase at equally spaced quantiles or quintiles. Several durations, from the daily change of a given climatic factor to that of confirmed meningitis cases, were tested.

A linear threshold parameterization (t) was also performed to explore a health impact from given values of climatic factors. It was assumed that a linear effect was observed

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from one single value of the climatic factor greater than the threshold chosen and null below. The lag dimension was specified from 0 to five days. Several thresholds were tested from the median of each climatic factor.

The quality of the adjustment of the models was checked by inspection of the sum of partial autocorrelation function (first 30 lags), residuals, and adjustment of predicted data according to observed data. The choice of the final model was based on the Akaike information criteria and adjustment of predicted data according to observed data[E1].

E1. Akaike H. in 2nd International Symposium on Information Theory. Budapest, 1973.

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